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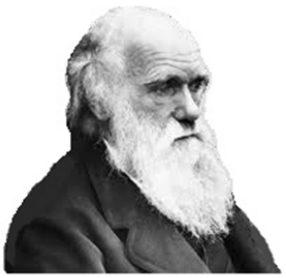

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Different approach to determination of pyrethroid pesticides in ornamental plants

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ABSTRACT

The aim of the presented research was to develop and optimize a methodology, particularly dedicated for the quantification of pyrethroids in ornamental plant material on the basis of a rose (*Rosa hybrid)* with the use of HPLC chromatography and QuEChERS extraction method. High repeatability and reproducibility of the results were obtained by using acetonitrile as an eluent. The determined limits of detection and quantification for deltamethrin equal 5.2 ng and 9.3 ng per 1 cm^3 of analysed solution respectively. For cypermethrin these values were: LOD 1.2 ng, LOQ 5.0 ng per 1 cm^3 of solution. It has been shown that solutions of deltamethrin and cypermethrin are of high stability – they can be stored at room temperature for as long as 28 days without a change in the concentration. The experiments presented showed that the QuEChERS extraction of deltamethrin from the tested samples can be performed with efficiency above 93% using acetonitrile as a solvent, magnesium sulphate and sodium acetate as the separation salts. For purification Supel ™ QUE sorbent by Supelco was successfully applied. The described analytical method may be a valuable and relatively cheap tool to control the amounts of these pesticides sprayed in environment, wherever there is a suspicion of their excessive use.

***Keywords*:** HPLC, cypermethrin, deltamethrin, QuEChERS, rose petals, *Rosa hybrid*

1. INTRODUCTION

There is no exaggeration in the sentence, that pesticides are one of the most important group of substances of human concern. On the one hand plant protection products are indispensable in modern agriculture and horticulture but, on the other hand, the lack of

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selectivity of most of them together with long time of biodegradation cause a great threat to human.

Pyrethroid pesticides are among the most popular insecticides. They are so-called third generation pesticides, which contain hormone analogues, chitin biosynthesis inhibitors, pheromones and attractants as active substances. Pyrethroids are equivalents of synthetic pyrethrins – a natural insecticide derived from chrysanthemum flowers (*Chrysanthemum cinerariafolium*). Insecticidal properties of pyrethrins were discovered in the 19th century and confirmed by further studies [1-3].

Pyrethroids and pyrethrins are esters of chrysanthemic acid and alcohol (in the case of natural pyrethrins, alcohols forming the ester molecules are: pyrethrol, cinerol and jasmonol). The chemical structure of natural pyrethrins is shown in Fig. 1. For over 20 years, these insecticides have been used to control the insects that threaten many species of cereals [4].

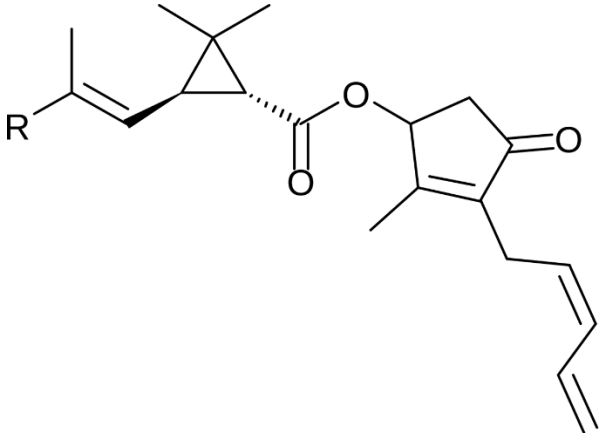

Figure 1. Chemical structure of natural pyrethrin. R is $–CH_3$ (Pyrethrin I) or $–CO_2CH_3$ group (Pyrethrin II).

In recent years they have replaced organophosphorus pesticides, which have been recognized as being among the most dangerous plant protection products and which are currently being phased out [5, 6]. Due to the low toxicity to mammals and birds, pyrethroid pesticides are nowadays widely used in agriculture, forestry, horticulture, and for the removal of insects inside buildings, such as houses or hospitals [7-9]. Moreover, pyrethrins and synthetic pyrethroids are even recommended by the World Health Organization for the disinfection of aircrafts [10]. Their effectiveness makes them practically irreplaceable in horticulture and agriculture [11]. Despite the high selectivity and the relatively short decomposition time of pyrethroid pesticides, they may be highly toxic to a number of species of beneficial insects (such as plants pollinators) fish, as well as for human. The problem is serious enough that the EU set the maximum residue levels of pyrethroid pesticides in food in the standards [12, 13].

The development of studies on the effects of pyrethroids on living organisms makes it necessary to improve and to specify analytical methods which allow their quantitative determination in particular environmental samples [14, 15]. There are at least a few methods used routinely to determine pesticides in different matrices, including plant material and food. Deltamethrin and cypermethrin may be determined for instance according to the European norm EN 15662:2018 [16]. The information concerning validation procedures for different groups of

pesticides can be found also in the in the document of European Commission SANTE/11813/2017 [17]. These techniques are widely used for the determination of many groups of pesticides [18-20]. However, difficulties have been noted, while implementing typical - universal procedures to the pesticide analysis, mainly due to the fact of different chemical structures of pesticides as well as different origin of samples studied [21, 22]. On the other hand, one can observe the development of sophisticated analytical methods that allow the determination of low concentrations of pyrethroids [23]. However, these methods, are in practice difficult to apply in typical laboratories mainly due to costs of analysis. Thus, one can notice the lack of relative simple methods dedicated to the particular pesticides and, in addition, adapted to the specific matrix. Such methods, optimized to the particular pesticide and matrix may serve both to conduct more demanding analyses, and as a reference to the general methods quoted earlier which involve the analysis of a broader spectrum of pesticides in a number of matrices.

The aim of this research was to verify the applicability of sample preparation method specially adapted to ornamental plants, based on the QuEChERS extraction (Quick Easy Cheap Effective Rugged and Safe) with the modified high performance liquid chromatography (HPLC) for the quantification of trace concentrations of pyrethroids. We postulate that a separate but simple and relatively inexpensive methodology has to be developed for each group of chemicals and types of samples independently from the general analytical methods described for instance in national and international standards. In this work we focus on the optimization of method for the determination of the most common representatives of pyrethroids: deltamethrin and cypermethrin (Fig. 2), in ornamental plants.

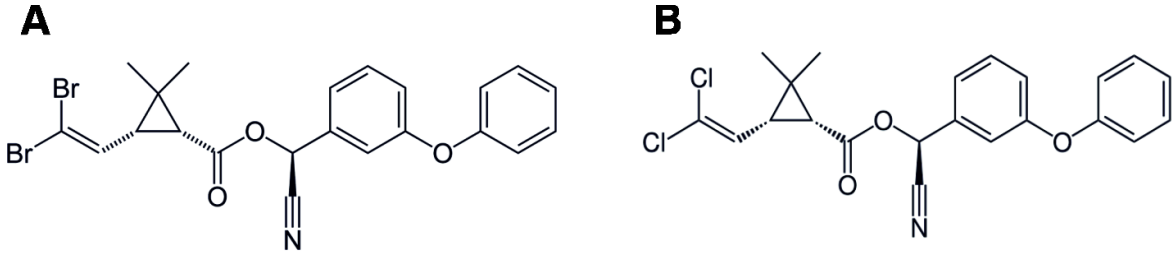

Figure 2. Chemical structures of deltamethrin (A) and cypermethrin (B).

2. MATERIALS AND METHODS

In order to measure the amount of pyrethroid pesticide in the samples of ornamental plants, all the steps of the technique of preparation based on the extraction method QuEChERS (Quick Easy Cheap Effective Rugged and Safe) were optimized. These included extraction of insecticides from the original sample, selection of suitable salts for the separation of inorganic and organic fractions and selection of the appropriate sorbent for purification by solid phase extraction (SPE) [24, 25]. Subsequently, the extraction efficiency of pyrethroid insecticides from plant material was checked.

Analyses of pesticides were made using a liquid chromatograph Dionex 3000 Ultimate with a photometric detector. By modifying the composition of the eluent and its flow rate, the measurement conditions were optimized and the calibration curves over a wide concentration range for both compounds were plotted. Reproducibility of the analytical method, the limit of

detection as well as the limit of quantification of the test substance and the stability of the standard solution used to calibrate the chromatograph were determined. The optimized method was used to determine the content of deltamethrin in a sample of a plant protection product Decis 2.5 EC® which is commercially available in many countries. Moreover, the applicability of the optimized analytical procedure for the isolation and identification of deltamethrin from rose petals after spraying flowers with Decis protection agent (in an amount recommended by the producer for use in gardening) was checked.

All the reagents used in the study were of HPLC purity. Isolation from the plant matrices was performed using acetonitrile, magnesium sulphate and sodium acetate. The extract was purified with a Supel ™ QUE sorbent (which contains zirconium oxide) bought from Supelco. Pesticides' standards were bought from Sigma Aldrich. The chromatographic analyses were carried out using acetonitrile as the eluent, water purified with Millipore system and AccuCORE C18 column (100 × 2.1 mm × 2.6 μm).

The plant material used for the study were potted miniature rose flowers (*Rosa hybrid*). Petals of rose from the local cultivation had been chosen as the experimental samples due to the fact that a number of commercially available insecticides containing deltamethrin are used in horticulture. Plant protection product Decis 2.5 EC® is commercially available and was bought in garden shop.

3. RESULTS AND DISCUSSION

3. 1. QUECHERS methodology – plant extract preparation

The extract from rose petals was prepared according to the following procedure. Initially the appropriate buffer salts: magnesium sulphate, and sodium acetate were selected. The best recoveries of the analyte were achieved using 0.4 g $MgSO_4$ and 0.2 g of CH_3COONa per 1 g of the sample of the plant material dissolved in at least 1 cm^3 of acetonitrile. The optimal amount of the sorbent Supel ™ Que was determined to be of 0.1 g per 1 g of sample. The extract of 3.0 g of crushed rose petals was prepared by adding a mixture of buffer salts (1.2 g magnesium sulphate and 0.6 g of sodium acetate), 4 cm^3 of acetonitrile, followed by mixing and centrifugation (6000 rpm for 10 minutes). 2 cm^3 of the extract was cleaned using Supel ™ QUE sorbent. The procedure described above was repeated three times.

3. 2. Determination of the conditions of chromatographic analysis

The first stage of the analysis was to develop the chromatographic conditions. In order to determine the optimal parameters of the analytical method, standard solution of deltamethrin in acetonitrile (with a concentration of 0.519 mg/ cm^3) was passed through a chromatography column and the analysis was performed. The width of the chromatographic peak in the half of its height was determined. The mixture of water and acetonitrile in a volume ratio of 1:1 was selected initially as the eluent. For flows of 0.50 and 0.75 cm^3/min, peak widths at half of height equalled 0.80 and 0.63 min respectively. Subsequently, gradient elution with increasing up to 80% content of acetonitrile was applied – this gave the peak width of 0.50 minute (at a flow rate of 0.75 cm^3 / min). In contrast, when isocratic mixture of water and acetonitrile mixed in the ratio of 2:8 was used with a flow rate of 0.60 cm^3/min, the peak width equalled 0.30 min. Finally, in order to shorten the time of analysis, eluent flow rates of 1.00, 2.00 and 2.50 cm^3/min were applied. It is noteworthy that there was a significant decrease in peak width at half height

using only acetonitrile as eluent – it amounted to 0.15, 0.02 and 0.02 min respectively. However, the decrease of resolution in case of flow rate 2.50 cm^3/min had been observed as well. Therefore the flow rate 2.0 cm^3/min of pure acetonitrile was chosen for the further studies. The studied analysis programs are summarized in Table 1.

Table 1. Chromatographic conditions tested for the determination of deltamethrin.

#	Flow [cm^3/min]	Type of elution	Time of analysis, [min.]	Composition		Peak width at half high, [min.]
				acetonitrile	water	
1	0.50	izocratic	20.0	50	50	0.800
2	0.75	izocratic	7.0	50	50	0.630
3	0.75	gradient	0.0	20	80	0.500
			7.5	80	20	
4	0.60	izocratic	5.0	80	20	0.300
5	1.00	isocratic	2.5	100	0	0,150
6	2.00	izocratic	1.1	100	0	0.020
7	2.50	isocratic	0.9	100	0	0,020

3. 3. Calibration of chromatographic analysis

Eighteen solutions of different concentrations of deltamethrin in acetonitrile were applied in order to prepare the calibration curve. The injections were performed subsequently. Each time the volume of 1.0 µl of every solution was injected and the appropriate area of the obtained peak was noticed. Fig. 3 presents the obtained calibration curve. The calibration parameters for cypermethrin were determinated in the similar way as in case of deltamethrin. The calibration curve is presented in Fig. 4. Due to the fact that the chromatographic method was applied to determine the content of deltamethrin in plant samples (rose extract), another calibration was performed with the plant extract prepared according to QUECHERS methodology, as a solvent for the standard substance. The mean efflux time for deltamethrin dissolved in the plant extract was approximately 0.03 minutes longer comparing to the efflux time for the model conditions described earlier in this chapter. Subsequently, ten vials were filled with ten different volumes of the deltamethrin solution (from 10 µl to 200 µl of the solution of concentration 4.74 mg/ cm^3) and made up to 1 cm^3 with the extract of rose petals. The pure extract, without the addition of deltamethrin, was tested as the control sample. The chromatographic analysis was performed twice for each sample. The injection volume was 1 µl in case of every sample. Additionally, four injections of the volume of 0.4 µl were done in order to obtain smaller peaks and broader range of calibration curve. Fig. 5 presents the final calibration curve prepared using plant

extracts. The linear range obtained was from 19 to 948 ng of deltamethrin. However, the slope of the calibration curve plotted on the basis of the results from the solutions prepared with plant extracts dropped almost three times comparing to the solutions prepared in pure acetonitrile.

This fact confirmed the need of using plant extracts for the preparation of standard solutions to rise the reliability of the results.

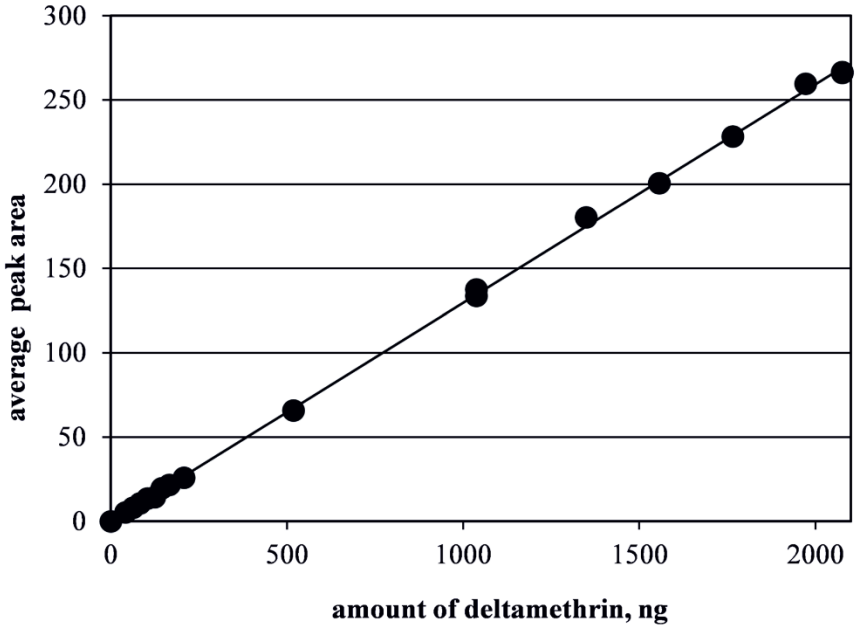

Figure 3. The calibration curve for deltamethrin, acetonitrile as a solvent ($y = 0{,}1292x - 0{,}3122$, $r^2 = 0.9997$).

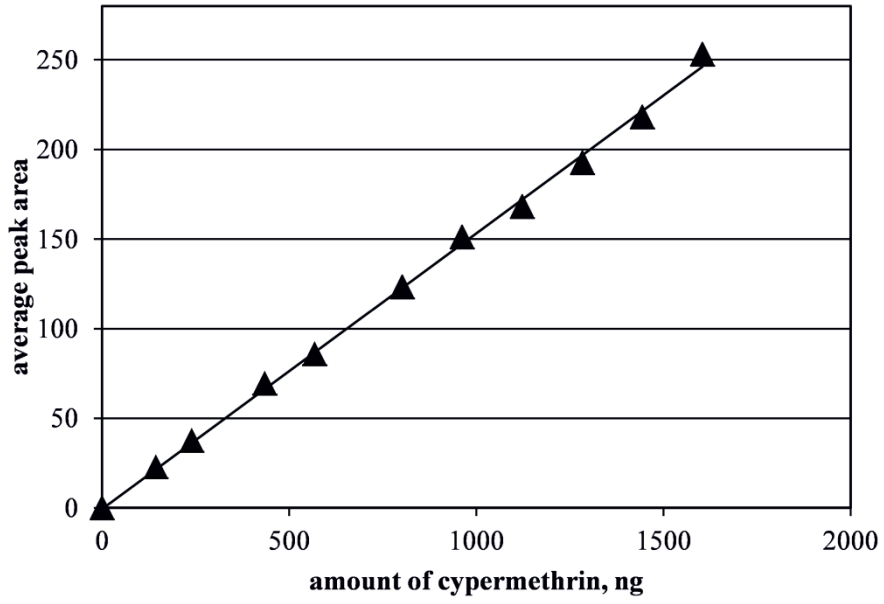

Figure 4. The calibration curve for cypermethrin using acetonitrile as a solvent, ($y = 0{,}1515x - 0{,}0108$, $r^2 = 0.9989$).

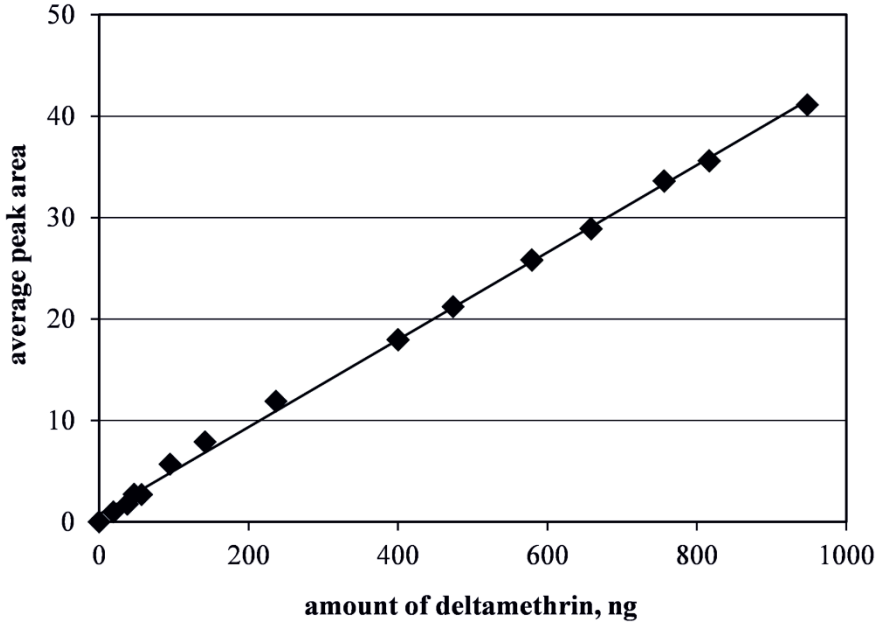

Figure 5. The calibration curve for deltamethrin prepared with a plant extract as solvent ($y = 0{,}0434x + 0{,}5894$, $r^2 = 0.9992$).

3. 4. Reproducibility of the method

The experiment presented below was designed to test the reproducibility of the analytical procedure, which consists of the extraction of pesticides from biological material and quantitative analysis of the extracted compounds. The experiment consisted of enrichment of biological samples (rose petals) with a known amount of deltamethrin, extraction and analysis using HPLC. Deltamethrin extraction from plant material was carried out using the QuEChERS technique described earlier. For this purpose, ten portions of crushed rose petals with a mass of 0.500 ± 0.009 g were prepared. Eight of them were enriched with deltamethrin solution and two were control samples. The amount of deltamethrin added to every batch of plant material was 0.402 ± 0.005 μg. Subsequently, to each portion of the plant material, a mixture of buffer salts (0.20 g $MgSO_4$ and 0.10 g CH_3COONa) and 4.0 cm^3 of acetonitrile was added. The mixture was vigorously shaken for 10 minutes. The solutions were centrifuged (6000 rpm for 10 minutes) and 2.0 cm^3 of each extract was transferred to a vial containing 0.050 g of sorbent Supel ™ QuE. The purified extracts were separated in chromatographic column according to the method described above and analysed using a photometric detector at a wavelength of 254 nm and 210 nm. Knowing the actual amount of deltamethrin added to the petals of roses, the percentage efficiency of recovery was calculated from the peak areas. The resulting recovery efficiencies were higher than 90% in every case and the average efficiency of recovery equalled $93.2 \pm 2.0\%$.

3. 5. Limits of quantification and detection

In order to determine the limits of quantification and detection, different amounts of deltamethrin were injected into the column, the areas of the peaks were determined and then

the ratios of signal to noise were calculated. It was found that the detection limit (defined as the concentration of the solution, for which the signal-to-noise ratio is 3.0) is equal to 5.2 ng of deltamethrin, while the detection limit (defined as the concentration of the solution for which the signal-to-noise ratio is 9.0) is equal to 9.3 ng. In the case of cypermethrin the limit of detection (LOD) and the limit of quantification (LOQ) were specified as well and equalled 1.2 ng / cm^3 and 5.0 ng / cm^3, respectively.

3. 6. Stability of the standard solution

To evaluate the compound stability, the solutions at a concentration of 163 μg/cm^3 of deltamethrin and of 401 μg/cm^3 of cypermethrin were used (the values are comparable to the concentration of working solutions used during agrotechnical treatments). The solutions of every pesticide were divided into two equal parts, one of which was stored at the room temperature and the other in a refrigerator – at 4 °C. The storage time was 28 days. Within that time, the contents of deltamethrin and cypermethrin were determined in different time intervals by using the analytical method described above. Table 2 presents the results of the analyses. The average values were the arithmetic average of three measurements taken after a particular storage time. Since the volume of the solution injected into the column was 20 μl, the amount of deltamethrin in the injection was 3.260 μg and cypermethrin 8.020 μg. On the basis of the results shown in Table 2 it can be stated that both the solution of deltamethrin and cypermethrin stored at 4 °C and at room temperature, have a high stability – the content of the analyte hardly changes for 28 days**.**

Table 2. Stability of the standard solutions of deltamethrin and cypermethrin - the average amount of substance and dispersion of the results [μg]

The average mass and the standard deviation of all measurements, [μg]			
delthametrin		cypermethrin	
the solution stored at room temperature	the solution stored at the temperature of 4 ºC	the solution stored at room temperature	the solution stored at the temperature of 4 ºC
3.216±0.034	3.174±0.036	7.896±0.046	7.819±0.065

3. 7. Determination of pyrethroid pesticides in environmental samples

To assess the possibility of using the developed analytical procedure for the determination of pyrethroids residues in environmental samples, a commercial plant protection product Decis® from Bayer, containing deltamethrin as an active substance, was used. First of all, the content of deltamethrin in this insecticide was determined. The obtained average value equalled 24.62±0.24 g/dm^3. This value differs only slightly from that specified by the manufacturer – 25.0 g/dm^3. Next, the laboratory simulation of spraying of Decis® was performed to model the

procedures performed in agriculture and horticulture. The solution was prepared according to the manufacturer's instructions – 125 µl of original product was taken to a volumetric flask of a capacity of 250 cm^3 and made up with water (the obtained concentration of the insecticide was of 0.05% vol, which is typical for spraying).

The resulting suspension was mixed and sprayed on the flower petals of laboratory cultivation of roses with an atomizer using the volume recommended by the manufacturer to protect ornamental flowers against harmful insects (which is defined as 6-15 dm^3 per 100 m^2). The flowers were spread over an area of about 4 m^2. After 24 hours, petals were collected randomly and separated into two groups – the inner and outer petals. Subsequently, all the collected petals were ground. Analyses were performed separately for the outer and inner petals of the flowers due to the assumption that the suspension did not penetrate inner petals as intensively as outer petals. Fourteen samples were weighed both for outer and inner petals – 0.5 g and 1.0 g per sample respectively. Extraction with QuEChERS technique was performed using the quantities of reagents described earlier and chromatographic analysis was performed in accordance with the developed procedure with rose extract as the solvent. For each extract at least two analyses were performed. Detection was performed at the wavelengths of 254 and 210 nm.

All the results obtained equalled above the limit of quantification. The average concentration of deltamethrin in the extract of the outer petals equalled 13.1 ±0.3 ng/cm^3 and in the extract of the inner petals 10.0 ±0.2 ng/cm^3. These values may be considered as save for pollinators and aquatic organisms, as the pesticide had been used strictly according to the imposed conditions. Based on this fact, it can be stated that if the procedure described above is followed, any excessive use of pyrethroids in crops can be detected.

4. CONCLUSIONS

The applied analytical conditions provide high repeatability of the results. It was proved that the high reproducibility of the results can be obtained by using acetonitrile as the eluent and the flow rate of 2.0 cm^3/min. Such chromatographic conditions provide very short analysis time, low solvent usage and still acceptable resolution. The limits of detection (LOD) and quantification (LOQ) were also determined. They equal for deltamethrin: LOD 5.2 ng and LOQ 9.3 ng and for cypermethrin: LOD 1.2 ng, LOQ 5.0 ng in 1 cm^3 of analysed solution. Moreover, it has been depicted that solutions of deltamethrin and cypermethrin are resistant to decay even when stored at the room temperature, for up to 28 days.

The presented experiments showed that the QuEChERS extraction of deltamethrin from the tested samples can be performed with efficiency as high as 93% using acetonitrile as a solvent, magnesium sulphate and sodium acetate as the separation salts. The determined optimal amounts of the reagents used for the extraction of pyrethroids from 1 g of plant material were as follows: C_2H_3N – at least 1 cm^3, $MgSO_4$ – 0.4 g, CH_3COONa – 0.2 g, Supel ™ QuE – 0.1 g and differ from the quantities recommended in the more general methods [16, 17]. After the simulation of spraying rose flowers in accordance with the procedure suggested by the producer of Decis®, the amounts of deltamethrin in the analysed extract (both in outer and inner petals) were still above the limit of quantification of the method. The described analytical method may therefore be a valuable tool to control the amounts of these pesticides sprayed in environment, wherever there is a suspicion of their excessive use.

However, the most important result of the research was the improvement, lowering of the costs and specifying both the methodology of plant sample preparation and chromatographic procedure of analysis of the pyrethroid pesticides. The results clearly show that in the studies of pesticides from different chemical groups such use of the customized analytical method should be considered, especially for the determination of trace amounts in different environmental samples.

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